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(54) Title: METHOD OF PRODUCING cis-9-OCTADECENOIC ACID COMPOSITIONS

(57) Abstract

A process for producing a high purity oleic acid composition utilizing enzymatic hydrolysis is disclosed. The process involves obtaining high oleic sunflower seed oil which oil contains triglycerides having fatty acid moieties of oleic acid in an amount of 60 % or more, preferably 80 % or more and further wherein the ratio of linoleic moiety to oleic moiety is less than about 0.25, preferably less than about 0.09. The oil obtained from the high oleic sunflower seed oils is subjected to enzymatic hydrolysis by contacting the oil with hydrolase enzymes and/or various combinations of hydrolase enzymes within an aqueous medium at a temperature in the range of 20-60°C and a pH in the range of about 4.5 to about 10. The oil, hydrolase enzyme and water are agitated so that hydrolysis occurs at the oil water interface and the acid moieties of the triglycerides are separated away. An oleic acid layer is allowed to form and separate away from the aqueous medium and the aqueous medium is then separated away to provide a highly pure oleic acid composition.

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METHOD OF PRODUCING cis-9-OCTADECENOIC ACID COMPOSITIONS

FIELD OF THE INVENTION

This invention relates generally to the field of methods for producing cis-9 octadecenoic acid, i.e. oleic acid. More particularly, the invention relates to the enzymatic hydrolysis of high oleic sunflower seed oil to produce a highly pure form of oleic acid as well as highly pure oleic acid compositions derived from such hydrolysis.

BACKGROUND OF THE INVENTION

Each year about one billion pounds of fatty acids are produced in the United States. About 80% of the fatty acids produced are derived from the industrial hydrolysis of tallow. (See M.W. Formo, in Bailey's Industrial Oil and Fat Products, 4th Edition, Volume 2, D. Swern, Ed., J. Wiley, New York, 1982, Page 379). Most fatty acids are produced by industrial fat splitting methods, however, there has been a recent increase in interest with respect to the use of enzymes in connection with the hydrolysis of fats in order to produce fatty acids. The main advantages of using enzymes as compared to conventional high-pressure steam for fat splitting are (1) a cleaner purer product due to a more specific reaction; (2) a lower energy requirement; and (3) the resulting sweet water is clearer,

i.e. the glycerin water mixture resulting from the hydrolysis is clearer. Oleic acid or cis-9-octadecenoic acid is a monounsaturated fatty acid present within natural fats and oils or biological lipids. Oleic acid is $CH_3(CH_2)_7(CH{:}CH)(CH_2)_7COOH$ and is also known as red oil. Oleic acid is a very important substance in both industry and biology. Cleaner, purer products are inherently safer when used in connection with products such as pharmaceuticals; and purer starting materials allow for the production of purer fine chemical derivatives.

Oleic acid is most generally obtained from high-pressure steam fat splitting processes using tallow as the starting material. When produced by such fat splitting processes, oleic acid is not generally obtained in a pure form. Highly purified oleic acid is both colorless and odorless and has excellent stability with respect to oxidative degradation. These properties make it extremely useful in connection with a large number of food and pharmaceutical products. Pure oleic acid can be used safely due to its excellent physical, chemical and physiological properties. Due to such properties oleic acid is actively and widely utilized in the fine chemical or specialty chemical fields. For example, oleic acid is extensively used in pharmaceuticals, cosmetics and foods and has found application in biochemical areas in connection with biosensors and biosurfactants. Oleic acid has also found application in connection with electronics for the stimulation of biological function as well as a number of other quickly developing high technology fields.

Many uses for oleic acid require that the oleic acid be very pure, and commercially available oleic acid generally includes fatty acid homologs having different carbon numbers and double bond numbers. In addition, commercially available oleic acid often contains various minor impurities. Oleic acid compositions which are impure have properties and characteristics which make them

safety and the like making such compositions incapable of performing adequately in a number of high technology applications.

Chemical and physical processing steps in a method of producing highly purified oleic acid has been disclosed within U.S. Patent 4,601,856 issued July 22, 1986. However, a number of more conventional oleic acid purification processes are disclosed in Bailey's Industrial Oil and Fat Products, Vol 2, 4th Ed. John Wiley & Sons, N.Y. 1982 (p.379-387). The processes disclosed in Bailey's are those most likely to be presently used commercially.

It has been indicated above that enzymes can be utilized in order to produce fatty acids from triglycerides and that the hydrolytic reactions resulting from the application of such enzymes to triglycerides can find application within fields of high technology. For example, the quantitative determination of mono-, di- and particularly triglycerides in the body fluids of man has been used in the clinical diagnosis of many diseases or disorders such as arteriosclerosis, diabetes mellitus, nephrosis, biliary obstruction and various metabolic derangements due to endocrine disturbances. Clinical analysis generally requires that the glycerol esters first be hydrolyzed to liberate glycerol and the corresponding fatty acids. In connection with such techniques an enzyme composition found to be useful for glycerol ester determination is disclosed within U.S. Patent 4,056,442. The patent discloses a composition useful for hydrolysis in an aqueous medium comprising a mixture of from 15 to 95 units of Rhizopus arrhizus lipase and from 5 to 85 units of Candida cylindracea lipase per 100 units of total lipase present.

A specific method and composition for the hydrolysis of triglycerides is disclosed within U.S. Patent 4,259,440 issued March 31, 1981 to Miles Laboratories Incorporated. The method includes the steps of adding lipase and

cholesterol esterase to a triglyceride in combination with a glycerol assay system and determining the amount of triglycerides present based on the amount of glycerol produced. Other patents which refer generally to the enzymatic hydrolysis of triglycerides are referred to within patent number 4,259,440.

Since one of the potential disadvantages of carrying out hydrolysis with the use of enzymes is cost, enzyme techniques have been developed which involve the immobilization of the enzyme on a substrate. U.S. Patent 4,275,011 discloses a process for the interesterification of oils and fats comprising treating such oils and fats with a water-soluble microbial enzyme. The microbial enzyme is absorbed on an inert, powdered, water insoluble dispersing agent. Thereafter, the enzyme which is absorbed onto the inert substrate is recovered from the reaction medium.

SUMMARY OF THE INVENTION

A method of producing a high purity oleic acid by the enzymatic hydrolysis of high oleic sunflower seed oil is disclosed. The method involves obtaining sunflower seed oil which is extremely high in its oleic content. Sunflower seed oils contain triglycerides. The high oleic sunflower seed oils such as used in connection with the present invention have an oleic content of 60% or more, more preferably 80% or more, still more preferably 88% or more, and most preferably about 95%. Such high oleic oils are subjected to enzymatic hydrolysis by contacting the triglycerides with a hydrolase enzyme to provide a

reaction product which includes a high purity oleic acid composition. The reaction medium resulting from the enzymatic hydrolysis contains the oleic acid, glycerol, and a number of contaminant acids. By carrying out the reaction in an aqueous medium the glycerol and other water soluble compounds can be easily separated from the water insoluble oleic acid.

In connection with the invention the terms "high oleic sunflower seed oil," "high oleic sunflower oil" and "high oleic oil" will be used synonymously to mean an oil extracted from the seed of a sunflower plant which oil contains triglycerides which have fatty acid moieties and wherein 60% or more of such moieties are oleic acid moieties (preferably 80% or more, more preferably 88% or more, most preferably about 95%) and further wherein the ratio of any linoleic acid moieties to oleic acid moieties is 0.25 or less, i.e. oleic moieties : linoleic moieties is 1:(0.25 or less), preferably 1:0.09 or less and most preferably 1: (0.09-0.01).

Further, the terms "high purity oleic acid compositions" and the like refers to oleic acid compositions obtained by using a "high oleic sunflower oil" starting material and carrying out the processing as disclosed and described herein in connection with the present invention. A typical high purity oleic acid composition of the present invention would have approximately the following physical characteristics:

	Typical Sample
Specific Gravity (at 15.6°C)	0.899
Color (ASTM)	L2.0
Color (Gardner)	5-6
% H_2O	0.13
Acid value	201
Iodine Value	87.8
Titer	18°C

These physical parameters will vary somewhat based on the oleic content of the starting oil.

A primary object of the present invention is to provide a method for producing a high purity oleic acid composition by the enzymatic hydrolysis of high oleic sunflower seed oil.

Another object of the present invention is to provide such a method for the enzymatic hydrolysis of high oleic sunflower seed oil whereby different enzymes are utilized to efficiently remove all the oleic acid from the triglycerides within the sunflower oil.

An advantage of the present invention is that the hydrolysis of the triglycerides within the sunflower oils can be carried out in an energy efficient manner.

A feature of the present invention is that the reaction product resulting from the hydrolysis of the high oleic sunflower seed oil is a high purity oleic acid composition having a variety of uses within high technology fields.

Another feature of the present invention is that it combines technological advancements from the unrelated fields of (1) agricultural plant development; (2) biochemical enzymatic hydrolysis and (3) chemical engineering purification procedures respectively, by combining advancements in (1) plant breeding based on cytoplasmic male sterility techniques; (2) the use of specific lipases to hydrolyze the triglycerides of oils; and (3) chemical reactions and physical separation procedures specifically adapted for purifying oleic acid.

These and other objects, advantages and features of the present invention will become apparent to those skilled in the art upon reading the details of the materials, methods and uses as more fully set forth below. Reference being made to the accompanying general structural formulas and flow charts forming a part hereof wherein like symbols refer to like molecular moieties and steps throughout.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

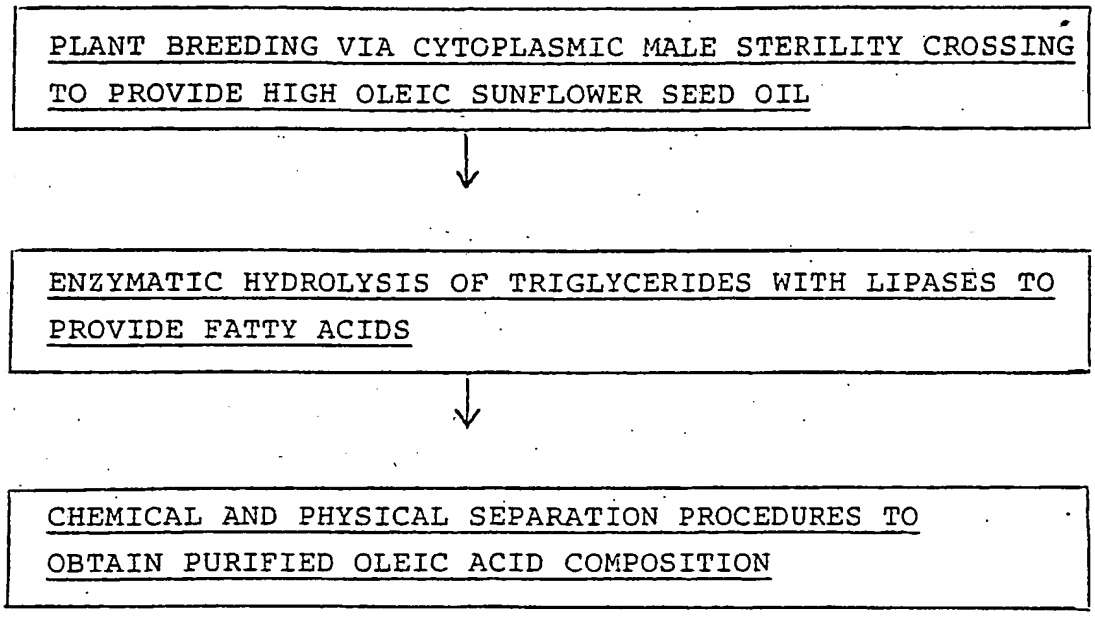
Before the present method for producing cis-9-octadecenoic acid (hereinafter oleic acid and the high purity oleic acid compositions) is disclosed and described, it is to be understood that this invention is not limited to the particular methods or compositions described as such methods and compositions may, of course, vary. It is also to be understood that the terminology used herein if for the purpose of describing particular embodiments only, and is not intended to be limiting since the scope of the present invention will be limited only by the appended claims.

It must be noted that as used in this specification and the appended claims, the singular forms "a", "an" and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a triglyceride" includes mixtures of triglycerides, reference to "an enzyme" includes reference to mixtures of enzymes and reference to "the hydrolysis" includes a plurality of hydrolysis reactions and so forth.

The present invention is a unique process which provides compositions of highly pure oleic acid by combining technological achievement in basically unrelated areas of science. Modern plant breeding technology is used to obtain seeds containing an oil of particularly high oleic content.

Highly efficient and selective enzymes are used to effectively remove fatty acids from the particular triglycerides of this high oleic oil in a manner which improves both yield and purity while carrying out the hydrolysis in an energy efficient manner. Chemical and physical purification and isolation techniques can then be applied to hydrolyzed triglyceride products to obtain a

highly pure oleic acid composition in a high yield. In order to describe and disclose the invention fully the three major aspects of the invention (shown in chart I below) will first be described individually.



1) PLANT BREEDING

The sunflower (genus Helianthus) is second only to the soybean as a source worldwide for vegetable oil. In the United States alone, there are approximately four million acres planted annually in sunflower, primarily in the Dakotas and in Minnesota. Average sunflower yields in the United States range from about 1200 to about 1400 kilograms per hectare, with the oil content from harvested seed averaging about 40 to 45% on a dry weight basis. Increasing both the oil content (as a percentage of total plant weight) and the yield of these sunflower plants are major objectives of plant breeding projects which the present invention utilizes as source material.

On average the last decade has seen a rapid expansion of the acreage of sunflower planted in the United States. This is due, in part, to the several important developments in the field of sunflower breeding and varietal improvement of sunflower plants. One significant development was the discovery of cytoplasmic male sterility and genes for fertility restoration. This discovery allowed for the production of hybrid sunflower plants. The hybrid produced utilizing this process was introduced in the early 1970's. Such sunflower plants showed a yield advantage of about 25% over the open-pollinated varieties along with improved disease resistance, greater uniformity in height and flowering. In addition, these hybrids had a greater degree of self-compatibility, which alleviates the dependency on high insect pollinator populations for good seed set. Sunflower plants develop utilizing cytoplasmic male sterility techniques are disclosed within U. S. Patent 4,627,192 which is incorporated herein by reference.

A description of cytoplasmic male sterility (CMS) and genetic fertility restoration in sunflowers is presented by Fick, "Breeding and Genetics" in Sunflower Science and Technology, pages 279-338 (J.F. Carter, ed. 1978) which is incorporated herein by reference for its disclosure of cytoplasmic male sterility breeding and genetic fertility restoration techniques in connection with sunflowers. The production of a particular sunflower hybrid using CMS is described within U. S. Patent 4,378,655 which is incorporated herein by reference for its disclosure of particular sunflower hybrids and means of producing such hybrids. Although cytoplasmic male sterility is now the technique of choice for producing sunflower plants with substantially non-functional pollen for subsequent use in producing hybrids, other methods, also described in U. S. Patent

4,378,655 are available. These methods include the use of complete or partial genetic sterility based on the presence of recessive genes and the application of chemical gametocides. Plants having a high level of self-incompatibility can also be used in a method for hybrid production.

Sunflower oil is comprised primarily of palmitic, stearic, oleic and linoleic acids, with oleic and linoleic accounting for about 90% of the total fatty acid content of the conventional sunflower seed oils. However, sunflower seed oil is known to contain 13 varieties of fatty acids including linoleic, oleic, palmitic, stearic, linolenic, palmitoleic, arachidic, margaric, palmitic and behenic. (See T. Cuprina et.al. The Relative Amount of Fatty Acids in Sunflower Oil of Certain Inbred Lines and in Hybrids of Sunflowers, Institute for Agriculture and Horticulture, Yugoslavia, 1983 incorporated herein by reference for its disclosure of the content of sunflower oil). The unsaturated acids have one, two or three free bonds, e.g. respectively oleic, linoleic and linolenic. Other acids such as stearic and palmitic are saturated.

It has been recognized that there was an inverse relationship between oleic and linoleic acid which was highly influenced by environmental factors, especially temperature during the growing season. The totals of the oleic and linoleic acid contents is generally about 90% of the acid content of the oil. Therefore, as the linoleic content increases to about 10% the oleic content decreases to about 80%. This relationship is merely a rule of thumb and should not be strictly construed. Cool northern climates generally yield higher linoleic acid-content sunflower seed, whereas high oleic acid values are more characteristic of seeds grown in warmer southern areas. A high linoleic acid concentration is desirable in sunflower

oil used in soft margarines and salad dressings, high oleic acid content is preferred for many other applications. For example, a high oleic sunflower seed oil is desirable with respect to the present invention which involves the production of a high purity oleic acid. The purity of the oleic acid, with respect to its lack of linoleic acid, increases the oxidative stability of the product obtained. As a consequence, the oxidative stability of conventional crude sunflower oil derived from seed grown in southern climates is nearly twice that of the crude oil extracted from northern-grown sunflower seeds.

In connection with the present invention, the terms "cultivar" and "variety" are used synonymously to refer to a group of plants (e.g., Pervenets) within a species (Helianthus Annuus) which share certain constant characteristics that separate them from other varieties within that species. A variety used in connection with the present invention may show overall variation between individuals within the variety, based primarily on Mendelian segregation of traits among the progeny of succeeding generations. A "line", as distinguished from a "variety" denotes a group of plants which display less variation among individuals, generally (although, not exclusively), by virtue of several generations of self-pollination. In addition, a "line" is defined for the purposes of the present disclosure, sufficiently broad to include a group of plants vegetatively propagated from a single parent plant using tissue culture techniques. The use of such lines to develop new hybrids is described within U. S. Patents 4,326,358 and 4,381,624, the contents of which are incorporated herein by reference for disclosure of such tissue culture techniques. A variety or a line is considered "true breeding" for a particular trait if it is genetically homozygous for that trait to

the extent that, when the true breeding variety or line is self-pollinated, a significant amount of independent segregation of the trait among the progeny is not observed.

As indicated above, the various fatty acids, such as stearic acid, oleic acid and linoleic acid, are characteristic of the oil of a given variety of seed. Such acid contents may be expressed as a percentage of the total fatty acid content of the trigylceride making up the oil. This method of describing the oils obtained from sunflower seeds used in connection with the present invention is utilized herein. For example, the dimensionless ratios of linoleic acid content to oleic acid content mentioned below are calculated by dividing the linoleic acid percentage of total fatty acid moieties on the triglyceride by the like percentage of oleic acid moieties. Thus, smaller numbers represent a larger percentage of oleic acid relative to linoleic acid.

In connection with the present invention, it is desirable to utilize an oil derived from a sunflower seed wherein the triglycerides of the oil have an oleic acid moiety content of greater than about 60% preferably 80% relative to the total fatty acid moiety content of the triglycerides present within the sunflower seed oil. The ratio of the amount of the linoleic acid in the seed to the amount of oleic acid in the seed is less than approximately 0.25, i.e. the ratio of oleic moiety to linoleic moiety is 1:0.25 preferably 1:0.09 or less. More preferably, the ratio of the amount of linoleic acid moiety in the seed to the amount of oleic acid moiety in the seed is in the range of about 0.01 to about 0.09, i.e. the ratio of oleic:linoleic is 1: (0.09-0.01).

As will be indicated below, it is possible to utilize enzymes which selectively remove particular fatty acids from the triglyceride of the oil. However, the selectivity of the enzyme is often not sufficiently specific to differentiate between linoleic and oleic acids containing the same number of carbons and an overlapping unsaturated position. Accordingly, it is particularly important to obtain an oil which has a dramatically lower linoleic content coupled with a high oleic content of 80% or greater by weight, and more preferably 88% oleic or greater and most preferably about 95% oleic content. It should be noted that in connection with the present invention, the sunflower seed oil is obtained from a substantially homogeneous assemblage of sunflower seeds. Any particular sunflower seed within the assemblage may well contain higher or lower amounts of oleic acid and different ratios of linoleic to oleic acid. However, the resulting statistical mixture of triglycerides obtained from the substantially homogeneous assemblage of sunflower seeds provides an oil which on average contains 60% or more oleic, preferably 80% or more, more preferably 88% or more oleic most preferably 95% or more oleic with the ratio of the linoleic to the oleic of less than 0.25, preferably less than 0.09 and more preferably in the range of about 1:(0.09-0.01).

Depending on the sunflower plant the oil is taken from the acid moieties of the oil will vary as will the relative amounts of those acid moieties. A typical oil used in connection with the present invention would include the following acid moieties in the given percent amounts:

Acid Moiety		% present
Oleic	(18 carbons, one double bond)	80.0
Linoleic	(18 carbons, 2 double bonds)	8.1
Stearic	(18 carbons, no double bond)	5.5
Palmitic	(16 carbons, no double bond)	4.2
Behenic	(22 carbons, no double bond)	0.7
Linolenic	(18 carbons, 3 double bonds)	0.2

In general, a variation ± 10% would be within the scope of the present invention.

As indicated above, it is possible to obtain such high oleic sunflower seed oil by breeding techniques as described in U. S. Patent 4,627,192 which is incorporated herein by reference for its disclosure of such breeding techniques. In addition, U. S. application number 769,502 filed August 26, 1985 is incorporated herein for its disclosure of sunflower breeding techniques as well as the oil obtained from the seeds yielded from such sunflowers.

Although the high oleic sunflower oil preferably used in connection with the present invention is most generally obtained from the breeding techniques as disclosed in U.S. Patent 4,627,192, such oil might be obtained from sunflower seeds produced by plants developed by other means. Accordingly, it should be pointed out that any sunflower seed oil having the oleic content and the ratio of linoleic to oleic acid as described above would be useful in connection with the present invention. Plants yielding such seeds might be obtained in a number of manners including the manipulation of plant material via tissue culturing, vector and transformation systems to insert genetic material into a target host plant cell, (e.g., Ti plasmid vectors, microinjection of genetic material, and cauliflower mosaic virus vectors) and gene isolation and characterization techniques.

2) ENZYMATIC HYDROLYSIS

As indicated above, there are a number of different methods for the hydrolysis of fats and oils which involve decomposition by saponification or acid hydrolysis. Such methods include the decomposition of the triglyceride by the application of high temperature and steam pressure and the Twitchell decomposition method. In general, the fatty acid compositions obtained utilizing such decomposition methods are not particularly pure as indicated by their

darker colors and the resulting sweet water is quite contaminated. Their impurity contributes to their oxidative instability and unsuitability for use in connection with many high tech applications. In order to purify the hydrolyzed products obtained from these techniques, it is necessary to carry out distillation steps which increase the amount of energy necessary to produce the final product.

The distillation steps required vary depending on the chain length of the fatty acid being isolated. As the chain length increases, the amount of temperature and vacuum required to carry out distillation also increases which further increases the expense due to the additional energy requirements. Further, as the temperature of distillation is increased, reactions can occur among the fatty acids themselves resulting in polymerization and the oxidative degradation. This decreases the yield of the fatty acid obtained from such techniques. In addition to the occurrence of polymerization reactions, some fatty acids isomerize at their double bonds creating large amounts of isomerized fatty acids which decrease the yield of the fatty acid product obtained.

Having noted the above problems, the present invention does not make use of any high pressure, high temperature techniques in order to separate the fatty acids from the trigylcerides within the oils. The present invention utilizes enzymatic hydrolysis to carry out decomposition of the fats and oils. The enzymatic hydrolysis reactions carried out in accordance with the present invention are very selective and have a very low energy requirement. The selectivity of the reaction increases the amount of a particular fatty acid removed from the triglyceride, thus, increasing the purity of the resulting yield. Further, since high temperatures are not required during the hydrolysis and are actually undesirable, fatty acids are not lost by polymerization or

isomerization reactions which occur under high temperature.

The ability of enzyme derived from specific microbes to hydrolyze a material is often specific to the material. Accordingly, the enzymatic hydrolysis reaction used in connection with the present invention are carried out only on high oleic sunflower seed oils which have been described above. To obtain the present invention, particular reaction conditions necessary to enzymatically hydrolyze such high oleic sunflower seed oil have been carefully studied with regard to the type and amount of enzyme, the pH of the reaction mixture, the type and amount of additives, the temperature and the amount of water necessary to obtain both a high purity oleic acid composition and a high yield. Adjustment of these parameters can increase the percent of hydrolysis and/or selectivity of the reaction.

In connection with types of enzymes, the enzymes used in connection with the present invention can be divided into different categories as follows:

(1) non-site-specific enzymes;
(2) site-specific enzymes; and,
(3) fatty acid selective enzymes.

Various enzymes and particular combinations of enzymes have been found to be particularly useful in connection with the present invention to obtain both high purity oleic acid in a high yield based on the amount of high oleic sunflower oil starting material.

High oleic sunflower seed oil used in connection with the present invention is comprised of trigylcerides having the following general structural formula (I):

$$\begin{bmatrix} OC(O)R \\ OC(O)R' \end{bmatrix}$$

wherein R, R' and R" are hydrocarbon moieties of the acid moieties, 80% or more of which are oleic acid moieties. As indicated above, preferably 88% or more of the acid moieties are oleic and most preferably about 95% are oleic moieties.

When a non-site-specific enzyme is brought into contact with a triglyceride of general structural formula (I), the enzyme will separate all of the fatty acid moieties at all three positions and leave a mixture of glycerol and the separated fatty acids. When a site-specific enzyme is utilized in connection with the triglyceride, the site-specific enzyme generally removes the fatty acid moiety from the two primary positions of the triglyceride. Thus, a 100% efficient reaction of such a site-specific moiety with such a triglyceride would remove two-thirds of the fatty acid moieties. When a fatty acid-specific enzyme is reacted with the triglyceride, the enzyme will react with fatty acid positions wherein particular fatty acids are located (the acid generally being recognized by a particular unsaturated position). For example, the enzyme could react with only oleic fatty acid moieties which have an unsaturated position at the delta nine carbon. However, such fatty acid-specific moieties might also react with other non-oleic moieties which also have an unsaturated position at the ninth carbon.

In connection with the present invention, it was first discovered that the yield and purity of the oleic acid product obtained could be increased by utilizing the plant breeding techniques described above to provide a high oleic sunflower seed oil wherein the triglycerides had a particularly high amount of oleic moieties. Thereafter, differences in various enzymes and reaction conditions were studied to make the best possible use of the best possible starting material. This was done by varying the enzymes and combining different enzymes in

different manners at various pH's, additive amounts and amounts of water. Accordingly, one embodiment of the invention involves the use of the high oleic sunflower seed oil starting material in combination with enzymatic hydrolysis techniques to obtain a high yield of oleic acid in a relatively high purity. By carrying out the enzymatic hydrolysis at a water/oil interface, the resulting hydrolyzed product is comprised of fatty acids and glycerol with the glycerol being soluble within the aqueous phase. The aqueous phase is then separated away, leaving a relatively high yield of a high purity oleic acid composition.

It is possible to use different types of enzymes in connection with the present invention, by combining the enzymes or using them step-wise to obtain yields in step-wise reactions. In terms of biochemical nomenclature enzymes are divided into six groups. The present invention involves the use of hydrolase enzymes, and more specifically water soluble lipases.

The following is a listing of microbes from which are derived non site-selective lipases used in connection with the present invention:

Candida rugosa (cylindracae)
Chromobacterium viscosum
Humicola lanuginosa
Candida lipolytica

The following is a listing of microbes from which are derived site-selective enzymes used in connection with the present invention:

Aspergillus niger, Mucor miehei, Mucor pusilis
Rhizopus sp. Pseudomonas sp.
Penicillium cyclopium.

Geotrichum candidum microbes are the source of enzymes which are selective for fatty acids with a delta nine carbon atom.

Some particular combinations of enzymes are found to be particularly useful in connection with the present invention are derived from the following combinations of microbes.

1) Candida rugosa/Penicillium cyclopium
2) Aspergillus niger/Penicillium cyclopium
3) Mucor miehei/Candida rugosa/Penicillium cyclopium
4) Mucor pusilis/Penicillium cyclopium
5) Chromobacterium viscosum/Penicillium cyclopium
6) Mucor miehei/Penicillium-cyclopium
7) Pseudomonas sp./Penicillium cyclopium

In addition to microbial enzyme sources, animal sources may be used in connection with this invention such as Porcine pancreatic lipase, Bovine pancreatic lipase and Porcine liver esterase.

In connection with the enzymatic hydrolysis reactions carried out in connection with the present invention, the amount of the enzyme utilized depends on the amount of the triglyceride to be hydrolyzed. The amount of the enzyme is expressed in units (U) of activity in connection with the hydrolytic decomposition of the triglyceride. The amount of enzyme utilized varies depending on the particular enzyme used. Since the present invention only applies enzymes to high oleic sunflower seed oils, the amount and type of enzyme does not vary substantially depending upon the oil being hydrolyzed. However, other considerations do effect the amount of the enzyme utilized such as the reaction time, temperature and pH of the reaction medium. In connection with the present invention, it is useful to utilize 10 to 5,000 preferably

10 to 100 more preferably 20 to 40 units per gram of high oleic sunflower seed oil.

In order to demonstrate how the amount of enzyme used varies with enzyme source, some ranges on the amounts are suggested below:

1) Candida rugosa: 7-10 U/m.eq. 23.8-34.0 U/g.
2) Porcine pancreatic: 100-1000 U/m.eq. 340-3,400 U/g.
3) Geotrichum candidum: 25 U/m.eq. 85.0 U/g.
4) Pseudomonas: 34 U/g.
5) Penicillium: 1 U/m.eq. 3.4 U/g.
6) Aspergillus niger: 10-100 U/m.eq. 340 U/g.

When used in commercially acceptable processes less enzyme is generally used. Some preferred ranges for some specific enzyme sources are as follows:

1) Candida: 23.8-34.0 U/g.
2) Porcine: 340 U/g.
3) Geotrichum: 85.0 U/g or less.
4) Pseudomonas: 34 U/g.
5) Mucor: 85.0 U/g or less.
6) Aspergillus: 34 U/g or less.

The enzymatic hydrolysis reaction carried out in connection with the present invention is preferably carried out in the presence of water in an amount in the range of about 0.5 to 1.5 times the amount of the high oleic sunflower seed oil. The water must be present in a sufficient amount to allow for the hydrolysis to efficiently proceed. However, the inclusion of too much water can also decrease the efficiency of hydrolysis and make it difficult to effectively remove and dispose of the aqueous phase of the reaction medium. The enzymatic hydrolysis reactions of the present invention take place at the

The relative amounts of oil and water varies with each system and must be adjusted in order to obtain the best results. In general the water/oil ratio is (1-½):(1-1½) preferably 1:(1-1½) at a temperature which is most preferably about 35°C ± 2°C. The temperature is generally about 38°-40°C with 1, 3 specific enzymes, more generally the temperature can range from 20°C to 60°C.

The pH of the enzymatic reaction mixture affects the hydrolysis. The hydrolytic reaction yields a carboxylic acid which is not water soluble. However, it may be desirable to include a sufficient amount of a buffer to control the pH within the range of about 4.5 to about 10.0, more preferably, 5.5 to 9. Further, the reaction can be carried out in the presence of other additives although other additives are not generally useful in connection with the present invention.

The enzymatic hydrolysis of the present invention is preferably carried out in a temperature range of about 20°C to 60°C, more preferably, about 30°C to 50°C. The temperature must generally be kept above 20°C in order to allow for the reaction to proceed quickly enough to economically carry out the procedure and must be carried out below 60°C in order to avoid deactivation of the enzyme prior to its interaction with the trigylceride. It is also desirable to continually agitate the reaction mixture in order to promote the enzymatic hydrolysis of the triglycerides.

The relative amounts of oil/water vary with other factors such as the amount of agitation, temperature and the enzyme source. Clearly, the amount of oil/water interface is affected by agitation and to some extent by temperature. The oil to water ratio is preferably 1:(1½), (more preferably 1: 1.2) the temperature is preferably 30°C to 50°C and agitation is generally carried out at sufficient speed in order to keep the oil and water phases in a homogeneous dispersion.

The following examples are provided so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make the oleic acid compositions and carry out the hydrolysis reactions of the invention and are not intended to limit the scope of what is regarded as the invention. Efforts have been made to insure accuracy with respect to numbers used (e.g. amounts, temperature, etc.) but some experimental errors and deviation should be accounted for. Unless indicated otherwise, parts are parts by weight, temperature is in degrees C, and pressure is at or near atmospheric.

EXAMPLE 1

Add 35ml of distilled water to 1,000 units of the lipase (10 U/m.eq. oil) obtained from Candida rugosa. (Amano Int. Enzyme Co.) Then add 100 m. eq. of high oleic sunflower seed oil. Allow the mixture to react at a temperature of about 35°C to 40°C for about 22-24 hours with stirring. Allow the mixture to settle and form two layers. Remove the lower layer containing water, glycerol and other water soluble contaminants to obtain an oleic acid composition. Hydrolysis should be in the range of about 88% to 94%.

EXAMPLE 2

Repeat the steps of Example 1 except add an acetate buffer to the water to maintain the pH at about 5.5 during reacting. Hydrolysis should be comparable with that of EXAMPLE 1 i.e., about 88% to 94%.

EXAMPLE 3

In 35 ml of water is dissolved 1000 U of Candida rugosa and 100 units of Penicillium cyclopium. Then add 100 m. eq. of high oleic sunflower seed oil containing 80% or more oleic acid moieties. Allow the mixture to react at a temperature of about 35°C to 40°C for about 6 hours with stirring. Allow the mixture to settle and form two layers. Remove the lower layer containing water, glycerol and other water soluble contaminants to obtain an oleic acid composition. The hydrolysis will rise to above 90% in 6 hours and if continued the hydrolysis will rise above 98% at 24 hours.

EXAMPLE 4

In 35 ml of water is dissolved 1000 U of Mucor miehei and 100 units of Penicillium cyclopium. Then add 100 m. eq. of high oleic sunflower seed oil. Allow the mixture to react at a temperature of about 35°C to 40°C for about 22-24 hours with stirring. Allow the mixture to settle and form two layers. Remove the lower layer containing water, glycerol and other water soluble contaminants to obtain an oleic acid composition. Hydrolysis should be above 96%.

EXAMPLE 5

Add about 70ml of distilled water to 200 units of the lipase obtained from Penicillium cyclopium and 2,000 units of the lipase obtained from the Pseudomonas (10 U/m.eq. oil). Then add 200 m. eq. of high oleic sunflower seed oil containing 80% or more oleic acid moieties. Allow the mixture to react at a temperature of about 35°C to 40°C for about 22-24 hours. Allow the mixture to settle and form two layers. Remove the lower layer containing water, glycerol and other water soluble contaminants to obtain an oleic acid composition. Hydrolysis should be above 93%.

EXAMPLE 6

Add about 105 ml of distilled water to 1,000 units of the lipase obtained from Candida rugosa; 1,000 units of the lipase obtained from Mucor miehei and 300 units of the lipase obtained from Penicillium cyclopium. Then add 300 m. eq. of high oleic sunflower seed oil. Allow the mixture to react at a temperature of about 35°C for about 22-24 hours at a pH in the range of about 5.5. Allow the mixture to settle and form two layers. Remove the lower layer containing water, glycerol and other water soluble contaminants to obtain an oleic acid composition. Hydrolysis should be in the range of about 80 to 95%.

EXAMPLE 7

Add from 10 to 100ml of distilled water to lipase obtained from Candida rugosa . Then add from 10 to 100 m. eq. of high oleic sunflower seed oil. The lipase should be added so as to provide 10 to 35 units of lipase per gram of oil. Allow the mixture to react at a temperature of about 35°C for about 4-24 hours. Allow the mixture to settle and form two layers. Remove the lower layer containing water, glycerol and other water soluble contaminants to obtain an oleic acid composition.

PHYSICAL AND CHEMICAL PURIFICATION

In accordance with the above disclosure relating to "plant breeding", a high oleic sunflower seed oil is obtained from a substantially homogenous assemblage of seeds. The high oleic sunflower seed oil as defined above includes triglycerides wherein the oleic moieties make up at least 60% of the fatty acid moieties on the triglyceride. Further, the resulting high oleic sunflower seed oil has a specifically defined ratio amount of linoleic to oleic acid. By utilizing this high oleic sunflower seed oil in connection with the hydrolysis procedures described above under "enzymatic hydrolysis" it is possible to obtain a hydrolysis reaction product which is very high in oleic acid content. The specific purity of the oleic acid varies somewhat depending on the starting high oleic sunflower oil used. The oleic acid composition derived from utilizing the high oleic sunflower seed oil and subjecting the oil to hydrolysis to

obtain oleic acid in accordance with the above description is considered to be the broadest aspect of the present invention. However, this oleic acid composition obtained from the high oleic sunflower seed oil can be further purified by physical and chemical procedures which will now be described in detail.

Firstly, after carrying out enzymatic hydrolysis the reaction product separates away into two phases with the upper phase being comprised of the fatty acids removed from the triglycerides and the lower aqueous phase being comprised largely of water having dissolved therein glycerol and certain contaminants from the high oleic sunflower seed oil. Accordingly, the first step in purification of the enzymatic hydrolysis product is to separate away the lower aqueous phase containing the water having the water soluble compounds therein. The remaining upper phase will contain a very high concentration of oleic acid.

The characteristics of the oleic acid composition will vary depending on the starting sunflower seeds used. The following is typical of such a high oleic acid composition:

Physical characteristics

Specific gravity (at 15.6°C)	0.899
Color (ASTM)	L2.0
Color (Gardner)	5-6
% H_2O	0.13
Acid value	201
Iodine Value	87.8
Titer	18°C

Chemical characteristics

Oleic acid 80%, linoleic acid 8.1%, stearic acid 5.5%, palmitic acid 4.2%, behenic acid 0.7% and linolenic acid 0.2%. The oil might also include some metals such as Ca, Bu, Zn, and Fe in small amounts, e.g. 1-100 ppm.

Each of the above physical and chemical characteristics might vary to different degrees, but in general might vary ± 10%.

From the above it is clear that the upper phase will include contaminant fatty acids such as linoleic acid as well as other fatty acids which having longer and/or shorter chains than oleic acid as well as greater and lesser degrees of unsaturation. These contaminant fatty acids can then be separated away by utilizing one or more chemical or physical separation techniques. For example, it is possible to use chemical separation techniques such as those described within U.S. Patent 4,601,856 incorporated herein by reference for its disclosure of chemical oleic acid purification techniques.

The high oleic acid composition of the invention is obtained as the upper phase resulting from the enzymatic hydrolysis process described above. This high oleic acid composition includes several different types of fatty acids and other contaminants as indicated above. It is possible to separate away fatty acids having different chain lengths and fatty acids having different degrees of unsaturation in order to further purify the oleic acid composition. More specifically, those fatty acids having more or less than 18 carbon atoms or more or less than one unsaturated bond can be separated away from the oleic acid which contains 18 carbon atoms and a single unsaturated bond.

A high oleic fatty acid composition of the present invention is winterized by subjecting the composition to low temperature treatments. By reducing the temperature gradually until crystallization begins, it is possible to separate away those fatty acids which contain higher degrees of saturation than oleic acid. Accordingly, by reducing the temperature gradually a point will be reached wherein fatty acids such as stearic and palmitic acid will

crystallize and precipitate within the composition. These fatty acids can then be removed to provide a further purified high oleic acid composition.

Polar solvents such as acetone and methanol allow saturated acids such as stearic acid and palmitic acid to crystallize almost quantitatively while the unsaturated acids such as oleic acid remain dissolved within a solvent. Accordingly, separation can be accomplished by including acetone and or methanol in the high oleic composition in an amount sufficient to bring about crystallization of the contaminant palmitic and stearic acids within the composition. After the crystallization occurs filtration can be carried out in order to remove the crystallized contaminant palmitic and stearic acids. In general the acetone or methanol solvents are added to the composition in a ratio of 3-4 liters of solvent per liter of fatty acid. After adding the solvent the temperature is reduced to bring about crystallization. In general the temperature is reduced to -10 to -15°C and filtration is carried out utilizing a vacuum rotary filter after crystallization occurs. The filter can then be sprayed with cold acetone to remove any free oleic acid. Solvents are removed from the oleic acid composition by flash evaporation and steam stripping.

In addition to the techniques referred to above it is possible to purify oleic acid compositions by chilling the fatty acid composition in water which contains a detergent such as sodium decyl sulfate. Crystals formed within the aqueous dispersion are coated with a film of detergent. These crystals remain in the water phase when the mixture is centrifugally separated. The oil phase is free of crystals and moisture.

It is also possible to purify high oleic acid compositions utilizing fractional distillation. Such

methods are described within U.S. Patents 2,054,096; 2,224,984; 2,322,056; and 2,674,570 all of which are incorporated herein by reference for their disclosure of fatty acid fractionation purification techniques.

EXAMPLE 8

First carry out the enzymatic hydrolysis procedure described within Example 1 above. After carrying out enzymatic hydrolysis separate away the lower aqueous phase and obtain the upper phase which includes the high oleic acid composition of the invention. Obtain approximately 1 liter of the oleic acid composition and add to the 1 liter of oleic acid composition 3 liters of methanol and reduce the temperature to a point in the range between -10 and -15°C. Maintain the reduced temperature until crystallization appears to be complete. Carry out filtration in order to remove the crystallized material and obtain a highly purified oleic acid composition.

The instant invention is shown and described herein in what is considered to be the most practical, and the preferred embodiments. It is recognized, however, that departures may be made therefrom which are within the scope of the invention, and that obvious modifications will occur to one skilled in the art upon reading this disclosure. It is further recognized that others may differ with respect to which particular embodiments of the invention are considered to be the preferred embodiments.

WHAT IS CLAIMED IS:

1. A process for producing an oleic acid composition, comprising the steps of:

obtaining a high oleic sunflower seed oil from an assemblage of sunflower seeds, the oil containing triglycerides wherein the triglycerides include fatty acid moieties comprised of 60% or more oleic acid moieties and further wherein the ratio of linoleic moiety to oleic moiety is less than about 0.25;

subjecting the high oleic sunflower seed oil to enzymatic hydrolysis by contacting the oil with a hydrolase enzyme in an aqueous medium under conditions which hydrolyze the triglycerides;

allowing an oleic acid layer to form and separate from an aqueous glycerol containing medium; and

separating away the aqueous glycerol containing medium to provide a high purity oleic acid composition.

2. The process as claimed in claim 1, wherein the triglycerides have a ratio of linoleic moiety to oleic moiety of less than 0.09.

3. The process as claimed in claim 1, wherein the seeds are a substantially homogeneous assemblage of seeds, the oleic acid moieties are present on the triglycerides in an amount of about 80% or more.

4. The process as claimed in claim 3, wherein the oleic acid moieties are present on the triglycerides in an amount of about 88%.

5. The process as claimed in claim 2, wherein the triglycerides have a ratio of linoleic moiety to oleic moiety in the range of from about 0.09 to about 0.01.

6. The process as claimed in claim 1, wherein the seeds are a substantially homogeneous assemblage of seeds, the oleic acid moieties are presented in an amount of about 95% and the triglycerides have a ratio of linoleic moiety to oleic moiety in the range of from about 0.09 to about 0.01.

7. The process as claimed in claim 1, wherein the enzymatic hydrolysis is carried out at a temperature in the range of from about 20°C to about 60°C at a pH in the range from about 4.5 to about 10.0.

8. The process as claimed in claim 7, wherein the temperature is in the range of 30° to 50°C and the pH is in the range of from about 5.5 to about 9.0.

9. The process as claimed in claim 1, wherein the enzymatic hydrolysis is carried out with a combination of hydrolase enzymes including a site-selective lipase and a non-site selective enzyme.

10. The process as claimed in claim 1, wherein the hydrolase enzyme is selected from the group consisting of a lipase derived from a Candida rugosa, Candida lipolytica, Mucor pusilis, Geotrichum candidum, Pseudomonas sp. and Penicillium cyclopium.

11. The process as claimed in claim 1, wherein the hydrolase enzyme is a lipase derived from a combination of Candida rugosa and Penicillium cyclopium.

12. The process as claimed in claim 1, wherein the hydrolase enzyme is a lipase derived from a combination of Aspergillus niger and Penicillium cyclopium.

13. The process as claimed in claim 1, wherein the hydrolase enzyme is a lipase derived from a combination of Mucor miehei, Candida rugosa and Penicillium cyclopium.

14. The process as claimed in claim 1, wherein the hydrolase enzyme is a lipase derived from a combination of Mucor pusilis and Penicillium cyclopium.

15. The process as claimed in claim 1, wherein the hydrolase enzyme is a lipase derived from a combination of Chromobacterium viscosum and Penicillium cyclopium.

16. The process as claimed in claim 1, wherein the hydrolase enzyme is a lipase derived from a combination of Mucor miehei and Penicillium cyclopium.

17. The process as claimed in claim 1, wherein the hydrolase enzyme is a lipase derived from a combination of Pseudomonas sp. and Penicillium cyclopium.

18. The process as claimed in claim 1, wherein the aqueous medium is substantially comprised of water having therein a buffer selected from the group consisting of acetate and phosphate buffers capable of maintaining the pH in the range of 4.5 to about 9.5.

19. The process as claimed in claim 18, wherein the water includes a pH buffer capable of maintaining the pH in the range of 5.5 to 9.0 during the enzymatic hydrolysis.

20. A process for producing an oleic acid composition, comprising the steps of:

subjecting a high oleic sunflower seed oil containing triglycerides wherein the triglycerides include fatty acid moieties comprised of 80% or more oleic acid moieties and further wherein the ratio of linoleic moiety to oleic moiety is less than about 0.25 to enzymatic hydrolysis by contacting the oil with a combination of a site-selective lipase and a non-site selective lipase in an aqueous medium at a temperature in the range of 30° to 50°C and a pH in the range of from about 5.5 to about 9.0 to hydrolyze the triglycerides;

separating away the aqueous medium to provide a high purity oleic acid composition.

21. The process as claimed in claim 20, further comprising:

adding a solvent selected from the group consisting of acetone and methanol to the high purity oleic acid composition;

reducing the temperature of the high oleic acid composition to a point in the range of about 0°C to -20°C;

maintaining the temperature in the range of 0°C to -20°C until crystallization of fatty acids occur; and

filtering to remove the crystallized fatty acids and provide a further purified oleic acid composition.

22. A process as claimed in claim 1, further comprising the steps of:

reducing the temperature of the high purity oleic acid composition to a temperature in the range of -10°C to -15°C;

maintaining the temperature in the range of -10°C to -15°C until fatty acid crystallization occurs; and

filtering in order to remove the crystallized

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 88/03480

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[4]: C 11 C 1/04; C 12 P 7/64

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[4]	C 11 C; C 12 P; A 01 H

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [9]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
A	EP, A, 0239470 (SOCIETE NATIONALE ELF AQUITAINE) 30 September 1987 see claim 8; page 4, lines 55-65; example 43 --	1
A	EP, A, 0232933 (AKZO) 19 August 1987 see claim 4; example 5; page 2, line 7 - page 3, line 10 --	1,7,8,10, 18,19
A	GB, A, 2176480 (KAO CORPORATION) 31 December 1986 see claim 1; example 1; page 5, line 62 - page 6, line 4 --	1,10
A	GB, A, 2188057 (INSTITUT PENYELIDIKAN MINYAK KELAPA SAWIT MALAYSIA) 23 September 1987 see claims 1,3; examples 2,10 --	1

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* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
9th February 1989	- 1. 03. 89
International Searching Authority	Signature of Authorized Officer
EUROPEAN PATENT OFFICE	P.C.G. VAN DER PUTTEN

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
P,A	EP, A, 0245076 (UNILEVER) 11 November 1987 see page 1, lines 46-53; examples 1-4 --	1-6
A	US, A, 4627192 (GERHARDT N. FICK) 9 December 1986 see claims 1-6; column 3, lines 21-23 cited in the application --	1-6
A	US, A, 4601856 (MASAO SUZUKI et al.) 22 July 1986 see claim 1; examples 1-5 cited in the application	1,20-22

ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO. US 8803480
SA 25149

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on 23/02/89
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		JP-A- 62232390	12-10-87
EP-A- 0232933	19-08-87	US-A- 4629742	16-12-86
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GB-A- 2176480	31-12-86	FR-A- 2583431	19-12-86
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		JP-A- 61287989	18-12-86
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